Veterinary Parasitology 196 (2013) 427–432

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

First insights into species and genotypes of *Echinococcus* in South Africa

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ARTICLE INFO

Article history:
Received 24 January 2013
Received in revised form 26 March 2013
Accepted 26 March 2013

Keywords:
Echinococcus granulosus
Cystic echinococcosis
Genotype
Human
Dog
South Africa

ABSTRACT

Cystic echinococcosis is a serious and neglected parasitic zoonosis that is regarded as an emerging disease world-wide. Effective control of the disease is based on understanding the variability of *Echinococcus granulosus* (sensu lato), as genotypic characteristics may influence lifecycle patterns, development rate, and transmission. No molecular epidemiological research has previously been conducted to shed light on genotypes responsible for the disease in South Africa. To identify strains circulating in the country, parasite material was collected from patients between August 2010 and September 2012 and analyzed by PCR/RFLP methods. A total of 32 samples was characterized as *E. granulosus* sensu stricto (G1–G3) (81%), *E. canadensis* (G6/7) (16%) and *E. ortleppi* (G5) (3%). Furthermore, two co-amplifying G6/7 genotypes were confirmed as G7 by sequencing. This is the first report on genotyping of *Echinococcus* species in South Africa, and, to the best of our knowledge, the first report of the G5 and G7 genotypes from humans in Africa.

1. Introduction

Echinococcus spp. are small cestodes, adult forms of which reside in the small intestines of definitive hosts (dogs and wild carnivores), while the larval (metacestode) stage develops in intermediate human and other animal hosts. The metacestode stage of certain species is responsible for cystic echinococcosis (CE) or hydatid disease, which is regarded as a serious parasitic zoonosis and an emerging disease world-wide (Budke et al., 2006). There are very few countries which have attempted to give true country-specific estimates of the incidence of CE; however, the global disease burden in humans, in terms of monetary losses and disability-adjusted life years (DALYs) is estimated to account for annual losses in excess of US $763,980,979 when taking into account underreported

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http://dx.doi.org/10.1016/j.vetpar.2013.03.033

cases, which is more than losses caused by onchocerciasis and almost similar to losses caused by African trypanosomiasis (Budke et al., 2006).

Intermediate hosts such as sheep, cattle, pigs and goats become infected when ingesting eggs from faeces of infected definitive hosts, and humans serve as accidental intermediate hosts. The disease is of veterinary and human public health importance, as infection can lead to severe illness and even death of intermediate hosts (Jenkins et al., 2005; Wahlers et al., 2012). Infection of animals has also been associated with serious losses of production to livestock industries (Cardona and Carmena, 2013).

The classification of the causative agents of CE has been a controversial subject for many years. Due to the limitations of morphological descriptions and lack of evidence for geographical or ecological segregation of the parasites, all were conventionally assigned to *Echinococcus granulosus* (Thompson and McManus, 2002; Thompson, 2008). The long-recognized diversity within this 'species' has led to the development of a strain concept, defined as variants that differ statistically from other groups of the same species in gene frequencies, and in one or more characters of actual or potential significance to the epidemiology and control of echinococcosis (Thompson and Lymbery, 1988). Eventually, 11 genetically distinct strains of *E. granulosus* (designated G1 to G10 and the 'lion strain') were described. These strains differ in characteristics that may affect lifecycle patterns, host specificity, development rate, pathogenicity, transmission dynamics, epidemiology and control (Eckert et al., 2001; McManus, 2002; McManus and Thompson, 2003; Hüttner and Romig, 2009). More recently, based on an increasing amount of genetic information, these 'strains' were assigned to a number of distinct species (*E. granulosus* sensu stricto, *E. equinus*, *E. ortleppi*, *E. canadensis* and *E. felidis*) (Nakao et al., 2010; Knapp et al., 2011). In any case, the proven diversity of the agents of CE requires detailed knowledge on the species and genotypes present in a certain area to allow targeted approaches in the development of effective control and prevention strategies.

The sheep strain (G1) of *E. granulosus* sensu stricto (*E. granulosus* s.s.) is the most epidemiologically important genotype with regard to its public health importance and geographic range (McManus et al., 1994; Breyer et al., 2004; Jenkins et al., 2005; Osman et al., 2009; Cardona and Carmena, 2013). In South Africa, the additional existence of *E. ortleppi* (G5), *E. equinus* (G4) and *E. felidis* (former lion strain) was known, but identification relied exclusively on the morphology of the adult worms in dogs or other carnivores as no non-molecular criteria exist to diagnose the metacestode stage in intermediate hosts, including humans (Ortlepp, 1934, 1937; Verster, 1965; Kumaratilake and Thompson, 1982; Hüttner et al., 2008). In South Africa, no molecular epidemiological studies have previously been undertaken to shed light on species and genotypes prevalent in the country. The geographically nearest country for which such information exists is Kenya, where the epidemiological situation is highly complex (involving at least four species of *Echinococcus*) and highly variable between regions of the country (Romig et al., 2011).

The aim of our study was to characterize species and genotypes of *Echinococcus* causing human CE in South Africa as a first step towards understanding the cestode biology and host species responsible for transmission of the disease in the country.

2. Materials and methods

2.1. Sample collection and demographic information

Between August 2010 and November 2012, excised cyst and aspirated cyst content were obtained from various sources around the country. The organs of cyst localization as well as geographic origin of patients was recorded when such information was available. Cyst samples were collected from CE patients admitted at two academic hospitals (Chris Hani Baragwanath Academic Hospital, Soweto; and Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg) in Gauteng Province, and sent to the National Institute for Communicable Diseases for molecular analysis. As part of efforts to increase recruitment of human CE samples, the National Health Laboratory Service's diagnostic laboratory information system (LIS) national data repository was interrogated repeatedly for positive CE results registered on the LIS, so that we were able to recruit samples from the relevant laboratories. Samples were also received from paediatric patients at two public hospitals (Cecilia Makiwane Hospital, Mdantsane; and Frere Hospital, East London) in the Eastern Cape Province. These were collected by general paediatric surgeons who performed thoracic or abdominal surgery on patients presenting with clinical CE symptoms.

In addition, cyst material from a cow was provided by meat inspectors at an abattoir based in Krugersdorp, Gauteng Province.

2.2. Ethical approval

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg, South Africa.

2.3. Microscopic diagnosis and organ involvement

To microscopically check fertility status of cysts, the hydatid fluid was centrifuged at 3000 rpm for 5 min; then the supernatant was poured out and a wet preparation was made from the sediment. The slides were viewed at 100× and 400× magnification using a light microscope. The cysts that contained hydatid hooklets or protoscolices were regarded as fertile, and all others were considered infertile.

2.4. DNA purification

All samples were preserved in 70% ethanol until DNA extraction. The DNA was extracted using a commercial extraction kit, QIAGEN DNA Mini Kit (QIAGEN, Valencia, CA, USA). Two different protocols were used, either a 'body fluid protocol' (for cyst fluid) or a 'tissue protocol' (for endocysts or protoscolices) following the manufacturer's instructions. For quality control, all samples were extracted together with a negative control (non-template water control) and all PCR amplifications were run with positive